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$T_{1\rho}$ relaxation can assess longitudinal proteoglycan loss from articular cartilage *in vitro*

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Summary

Objective: To assess the correlation between changes in spin-lattice relaxation in the rotating frame ($T_{1\rho}$) and proteoglycan (PG) loss from bovine articular cartilage and to demonstrate the feasibility of performing $T_{1\rho}$ MR imaging on a 1.5T clinical scanner.

Design: MR relaxation times ($T_{1\rho}$, T_2 and T_1) were measured from excised cartilage plugs (N=3) before and after two sequential digestions with trypsin on a 2T whole-body magnet. Proteoglycan and collagen loss induced by the trypsin digestion was measured using standard biochemical techniques. The correlation between changes in relaxation times and PG loss were tested with regression analysis. $T_{1\rho}$ MRI was also performed on a clinical 1.5T MRI system to determine whether the spatial distribution of PG loss could be detected. The MRI results were compared with histology sections of native and PG-depleted tissue.

Results: Increase in $T_{1\rho}$ relaxation times correlated with PG loss (R^2=0.81). $T_{1\rho}$ measurements alone were indicative of PG loss (R^2=0.8), the addition of T1 and T2 data into the statistical model did not improve the correlation substantially (R^2=0.83). $T_{1\rho}$-weighted imaging demonstrated a hyperintense lamina at the articular surface of the digested tissue, which was subjected to trypsin digestion that correlated with a superficial zone of PG loss observed on histological sections.

Conclusion: The results of this study demonstrate that $T_{1\rho}$ relaxation changes are correlated with PG loss *in vitro*. Furthermore, $T_{1\rho}$ measurements alone can be used to indicate PG loss data. $T_{1\rho}$ MRI may thus be developed into a useful adjunct to existing techniques for the evaluation of cartilage disease.

Key words: Magnetic resonance, Relaxation, Proteoglycan, Bovine cartilage.

Introduction

Osteoarthritis (OA) is a disease of articular cartilage degeneration. The early stages of OA are associated with a loss of proteoglycan (PG) from the extra-cellular matrix[1]. The recent advances in understanding the biology of OA has led to the development of several novel therapeutic agents. However, clinical trials have been hampered by the lack of non-invasive techniques to accurately assess the integrity of the tissue[2,3]. There is, therefore, a need for techniques that can non-invasively assess the integrity of articular cartilage[4].

Magnetic resonance imaging (MRI) affords excellent soft-tissue contrast and high spatial resolution[5]. For these reasons, MRI has been used to evaluate cartilage disease. Unfortunately, there have been no studies that have conclusively demonstrated the ability of conventional proton MRI to detect changes in the extra-cellular matrix[4]. Sodium imaging, and delayed gadolinium enhanced imaging (DeGEMRIC) have been the most promising techniques for assessing the biochemical integrity of cartilage[6,7]. Another promising method is spin-lattice relaxation in the rotating frame ($T_{1\rho}$) imaging of cartilage[8–10].

In our previous work[8], we have shown that $T_{1\rho}$ relaxation is altered following trypsin digestion but not after collagenase digestion. However, the extent of the correlation between PG loss and $T_{1\rho}$ increase was not demonstrated and remains unclear. The purpose of the current investigation is to determine whether serial $T_{1\rho}$ relaxation measurements can used to longitudinally assess the PG status of cartilage. We evaluate the correlation between spectroscopic measurements of T_2, $T_{1\rho}$ relaxation times and proteoglycan (PG) depletion from bovine articular cartilage by measuring the relaxation times as a function of sequential enzymatic digestion to deplete cartilage PG. We also show $T_{1\rho}$-weighted images obtained from bovine patellae after trypsin digestion to demonstrate the feasibility of using $T_{1\rho}$ on a clinical scanner to detect PG loss from cartilage.

Materials and Method

Bovine patellae were obtained from a local butcher shortly after slaughter of young cows between 1–2 years of age. The samples were rinsed in phosphate buffered

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saline (PBS) and stored on ice until MR examination was performed.

Nine mm diameter disks (N=3) were cored from the articular surface of three separate patellae and freed from the subchondral bone. The disks were then equilibrated in PBS. The samples were gently blotted dry and placed in a plastic holder for MR examination. These MR studies were performed on a 2T magnet interfaced to a custom-built console using a 1 cm diameter solenoid coil. The samples were placed in the coil with the articular surface normal to the direction of the main magnetic field. Relaxation times were measured with spectroscopic techniques, as previously described[8]. Spin locking was accomplished by using a 90° phase-shifted pulse, which immediately followed the initial excitation pulse. The spin-locked magnetization relaxes with a time constant $T_{1\rho}$, which is dependent of the amplitude of the spin-lock field (B_{SL}). T_2 was measured with a single echo spin-echo technique, and $T_{1\rho}$ was measured with a standard spin-lock sequence, with a B_{SL}=500 Hz. After the initial MR examination, the samples were removed from the magnet and placed in a 0.1 mg/ml trypsin solution in PBS at room temperature with agitation of 14/min. This trypsin digestion was performed for 1 h. The samples were then subjected to another MR examination. The samples were then further digested for another hour, in identical conditions. The MR protocol was repeated for another time. At the end of the final MR experiment, the tissue samples were finely minced and subjected to papain digestion, 500 μ/ml at 60°C overnight so that the tissue was completely digested.

Glycosaminoglycan (GAG) depletion was determined with the dimethyl methylene blue (DMMB) assay of Farndale *et al*.[11]. Collagen depletion was determined by measuring the hydroxyproline content according to the protocol of Bank *et al*.[12]. The digestion media resulting from each digestion were saved. The GAG and hydroxyproline assays were performed on each digestion aliquot. The GAG and hydroxyproline measurements were also performed on the media resulting from the final papain digestion. The total amount of GAG/hydroxyproline in the samples was calculated by adding the amount of GAG/hydroxyproline from each digestion point and that from the final papain digestion. With this data, we determined the fractional loss of GAG/hydroxyproline induced at each point during the sequential digestions.

For the imaging experiments, whole bovine patellae were used (N=4). For each patella the articular surface was bisected, and half of the tissue was subjected to trypsin digestion, the other half was kept in PBS, as previously described[13]. These specimens were then imaged on a standard 1.5T clinical scanner (GE Medical Systems). The imaging plane was scored with a scalpel blade for registration of MR images with histology sections. A $T_{1\rho}$-prepared fast spin-echo sequence[14] was used to obtain $T_{1\rho}$-weighted images, which were then used to calculate a $T_{1\rho}$ map. The following imaging parameters were employed, FOV=8 cm, 256×128 matrix, slice thickness=4 mm, TR=2 s, TE=22 ms, four echoes/excitation, two excitations/phase encode, spin-lock time (TSL)=10, 30, 50, 100, 130 ms, and a spin-lock amplitude (B_{SL}=500 Hz). $T_{1\rho}$ relaxation maps were calculated by performing a pixel-by-pixel fit of signal intensity vs TSL to a decaying single exponential function. $T_{1\rho}$-weighted and T_2-weighted images obtained with identical parameters were used for comparison purposes, T_2-weighted: TR=2 s, TE=112 ms, eight echoes/phase encode, and $T_{1\rho}$-weighted: TR=2 s, TSL=90 ms, and TE=22 ms with eight echoes/phase encode. The total spin evolution time (TE+TSL) was identical for both image sequences. The total imaging time (including set-up) for the proton imaging sequences was ~25 min. On two samples, sodium images were also acquired TR=20 ms, TE=2.4 ms, FOV=16 cm, 256×64 matrix, slice thickness=4 mm[15]. By comparing the signal strength of the cartilage to external standards placed next to the sample, we can obtain a measurement of the sodium content in the cartilage.

Signal intensities were measured for the $T_{1\rho}$-weighted and T_2-weighted images based on a region-of-interest (ROI) analysis (IDL Software RSI, Boulder CO). ROIs were placed to cover the thickness of the cartilage from the normal and PG depleted tissue. Signal ratios were then calculated.

Histology procedures

The tissue imaged in the MRI slice was dissected from the subchondral bone and placed in decalcifying solution (Cal-Ex, Fischer Scientific, U.S.A.) immediately after MRI examination. After 48 h of decalcification, the tissue was embedded in paraffin. Histological sections of 5 μm thickness were made and stained with hematoxylin&eosin and safranin-O&fast-green to demonstrate the morphology and PG, respectively.

SIMULATION OF THE SIGNAL FROM THE IMAGING SEQUENCE

The analytic solution for the signal level on the mth echo of a fast spin-echo sequence (90° flip angle) with 'n' echoes is given by[16], similarly, the signal from a $T_{1\rho}$-prepared fast spin echo sequence is given by:

$$S=S_o(1-\exp^{(-TR-mTE)/T_1})\exp(^{-nTE/T_2})\exp(^{-TSL/T_{1\rho}}) \quad (1)$$

where TSL is the spin-lock time, TR is the repetition time, TE is the echo time, T_1 is the longitudinal relaxation time, T_2 is the transverse relaxation time and $T_{1\rho}$ is the relaxation time in the presence of the spin-lock field. Using the above equations, we can compute the average signal intensity for a given set of relaxation values and pulse sequence parameters. We used the spectroscopic relaxation values measured on native and PG depleted tissue to estimate the signal strength from the $T_{1\rho}$ imaging sequence. Since $T_{1\rho}$ relaxation is highly dependent on the strength of the spin-lock field, and relatively independent of static field strength[17] relaxation times measured at 2T have been used to estimate the signal strength obtained at 1.5T.

STATISTICAL ANALYSIS

The reproducibility of relaxation measurements was determined by repeating the measurement at least three times and calculating the standard deviation on each sample. Propagation of error analysis was used to estimate the error bars associated with calculations of changes in relaxation time induced by PG loss[18]. The correlation between relaxation time changes and PG loss was determined with the Pearson product-moment correlation test (SigmaStat SPSS, Chicago, IL). The change in relaxation time induced by PG loss was evaluated by performing a linear regression analysis (Excel, Microsoft Co., Seattle WA). Linear regression analysis with clustering by tissue

sample was used to determine the correlation between relaxation times and PG loss (Stata, Stata Corp., College Station TX). The clustered linear regression analysis was used to examine whether relaxation measurements were indicative of PG loss, since we wish to know whether measured relaxation times can be used to longitudinally monitor PG loss. The independent variable was therefore the relaxation time and the dependent variable was PG loss. The statistical significance of signal differences observed on imaging measurements was determined by using a Student's *t*-test. A *P*-value<0.05 was considered to be significant. All values are reported as mean±standard deviation, unless otherwise stated.

Results

The total hydroxyproline content was slightly reduced after 1 h of trypsin digestion. The reduction in collagen content caused by 1 and 2 h of trypsin digestion was not statistically significantly (P=0.13). These data indicate that trypsin treatment did not cause a substantial decrease in the collagen content of bovine articular cartilage. The average PG loss was 32±9% after 1 h of degradation and 51±12% after 2 h of degradation. The uncertainty in assessing the PG loss is determined to be ±5% based on the reproducibility of the assay.

TISSUE EXPLANT EXPERIMENTS

$T_{1\rho}$ relaxation times increased as PG was depleted from the tissue (R^2=0.81, by Pearson Test [Fig. 1(a)]. The error bars represent the reproducibility of the $T_{1\rho}$ measurement (0.5%), as determined from repeat measurements on individual specimens. Since the aim of this study was to determine if $T_{1\rho}$ relaxation could be used to evaluate cartilage disease in a longitudinal fashion, we examined the correlation between the extent of relaxation time change and PG loss. The increase in $T_{1\rho}$ relaxation time was correlated with PG loss [Fig. 1(b)]. The $T_{1\rho}$ relaxation time of native tissue was 106.25±0.66 ms, indicating that the biological variability of the specimens, at baseline, was 0.625%. Linear regression of $T_{1\rho}$ against PG yielded a slope of 4.93 (P=0.032).

Elevated T_2 relaxation was also noted after PG loss, but was less correlated (R^2=0.65, Pearson). While T_2 increased after the initial degradation, T_2 changes were not consistent with subsequent PG loss. T_1 did not change substantially (<10%) as a function of PG loss. The T_2 of native tissue was 81.48±4.29 ms. The variability in T_2 of our samples was 5.1%. Linear regression of T_2 against PG yielded a slope of 2.64 (P=0.008). The majority of the variance in PG loss was reflected in the $T_{1\rho}$ measurements (R^2=0.8), the inclusion of T_1 and T_2 as other independent variables did not substantially improve the regression (R^2=0.83).

MRI EXPERIMENTS ON WHOLE PATELLAE

$T_{1\rho}$ imaging was also able to demonstrate the effects of trypsin degradation (Fig. 2). The sodium image (panel c) shows that the degraded portion has lower signal intensity, when compared to the normal portion. This change in signal intensity can be converted to sodium concentration. The control tissue has an average sodium concentration of 312 mM, while the degraded side has an average concentration of 274 mM. The $T_{1\rho}$-weighted image also demonstrates a 10% (P<0.001) increase in signal intensity on the degraded tissue when compared to the normal tissue. Based on the $T_{1\rho}$ signal expression simulations, we note that for a 15% loss of PG from the matrix we expect to see an 8% increase in the $T_{1\rho}$-weighted signal (Fig. 3). This is in good agreement with the measured value of 10%.

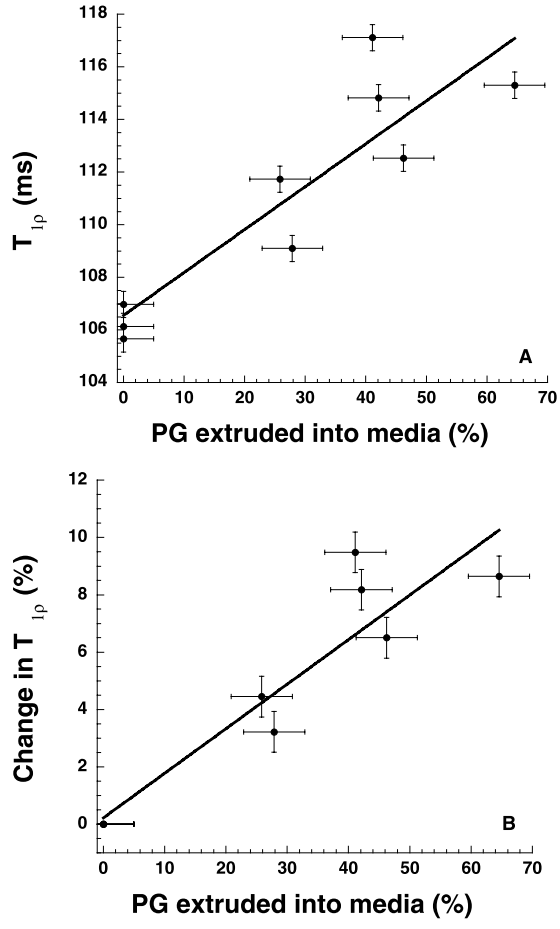

Fig. 1. Prolongation of $T_{1\rho}$ is correlated with the accumulation of PG in the digestion media. Panel (A) shows the correlation between the change in global $T_{1\rho}$ values with the accumulation of PG. Panel (B) shows the correlation between the percentage changes in global $T_{1\rho}$ values and PG accumulation in the media. Each PG loss data point was obtained by sequential digestion of a single cartilage plug. Our data suggests that $T_{1\rho}$ measurements may be useful for the longitudinal evaluation of cartilage disease. The typical error in the measurement of $T_{1\rho}$ was ±0.5%.

Figure 4 shows $T_{1\rho}$-weighted, T_2-weighted images and histological sections obtained from a patella that was treated with trypsin. Panels (C), (D) show the histologic sections stained with safranin-O and fast green, the PG depleted area is demarcated as the green superficial zone (black arrow). The articular surface is superior and the sub-chondral surface is inferior (open arrow). The $T_{1\rho}$-image demonstrates a clear hyperintense zone that appears to correspond to the region of PG depletion. The T_2-weighted image does not show a significant difference in contrast between the normal and degraded tissue.

Figure 5 shows a $T_{1\rho}$ map (panel A) and profiles through the thickness of degraded and native cartilage (panel B). The $T_{1\rho}$ map demonstrates a hyperintense lamina of the articular surface of the degraded cartilage, consistent with

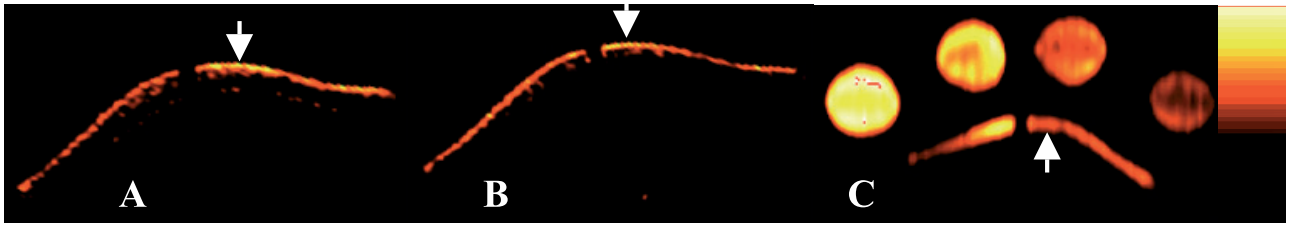

Fig. 2. $T_{1\rho}$-weighted imaging can detect the loss of PG from cartilage. Panel (A) shows a $T_{1\rho}$-weighted image, panel (B) shows a T_2-weighted image and panel (C) shows a sodium density image, the circular objects are phantoms that contain known concentrations of sodium ranging from 150–300 mM. The left half of the patella was degraded and the right half was the control tissue. The solid white arrow shows the hyperintense signal, relative to the contra-lateral side observed on the $T_{1\rho}$-weighted image but not on the T_2-weighted image after trypsin degradation. In this color scale, black depicts low signal intensity. The cartilage was digested with trypsin 0.2 mg/ml for 2 h. The PG loss is 15–20%, based on the sodium measurement and the biochemical assay. The signal difference on the $T_{1\rho}$-weighted image is 10%.

the hyperintense signal visualized on the $T_{1\rho}$-weighted MRI. The profile plots similarly show an increase in $T_{1\rho}$ in the articular region of the degraded cartilage.

Discussion

The main objective of the current work was to determine whether elevations in $T_{1\rho}$ relaxation are correlated with the loss of PG from cartilage. By performing sequential digestion experiments (which allow us to control for inherent biological variation) we find that $T_{1\rho}$ increases in a correlated manner with PG loss. Furthermore, $T_{1\rho}$-weighted imaging can be used to visualize the spatial variation in PG loss following trypsin digestion.

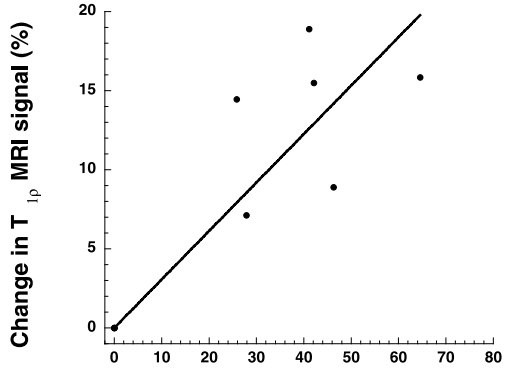

Fig. 3. The theoretically predicted increase in signal intensity as a function of PG accumulation in the media for the $T_{1\rho}$-fast spin echo sequence. The following parameters were used for the $T_{1\rho}$ simulations: TR=2 s, TE=22 ms, TSL=100 ms, the spectroscopic $T_{1\rho}$, T_1 and T_2 values were also used. The predicted change in signal intensity for the $T_{1\rho}$-weighted image is in good agreement with the observed values.

$T_{1\rho}$ is another magnetic resonance relaxation time that is sensitive to molecular interactions that have correlation times on the order of the reciprocal of the spin-lock frequency[19,20]. It has been proposed that $T_{1\rho}$ relaxation measurements may be useful for tissue characterization for this reason[21–23]. More recently, Virta *et al*. have shown that other interactions, in addition to simple molecular tumbling, may contribute to $T_{1\rho}$ relaxation in biological systems[24].

While the exact nature of $T_{1\rho}$ relaxation in biological systems remains unclear, it is known that any interaction that has a characteristic correlation time (τ) which satisfies the equality $\tau_c\ (\gamma B_{SL})^{-1}$ (where, γ is the gyromagnetic ratio of protons) can contribute to $T_{1\rho}$ relaxation. We postulate that interactions such as amide proton exchange[25], hydroxyl proton exchange[26], diffusion through inhomogeneous fields[27], or molecular motions[28] can all be $T_{1\rho}$ relaxation mechanisms. Further work is needed to clarify the exact contribution of each of these mechanisms to the overall $T_{1\rho}$ relaxation characteristics of cartilage.

Since $T_{1\rho}$ imaging requires the use of long pulses radio-frequency power deposition is a concern with clinical imaging. It is therefore advantageous to use low spin-lock fields when possible. Recent data has shown that human cartilage demonstrates significant $T_{1\rho}$ dispersion in the 0–375 Hz regime, *in vivo*[9]. Significant dispersion has also been reported in the same frequency range for bovine cartilage[8,10]. We have therefore used a B_{SL} value of 500 Hz in the present study to mimic clinically applicable imaging parameters.

The serial evaluation of cartilage before and after sequential trypsin digestions used in the present study allows us to directly compare the effects of PG loss on the

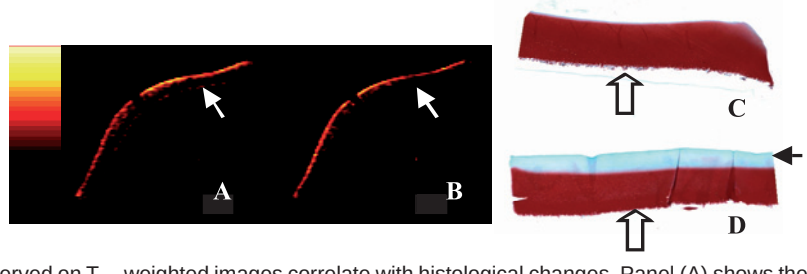

Fig. 4. Signal changes observed on $T_{1\rho}$-weighted images correlate with histological changes. Panel (A) shows the $T_{1\rho}$-weighted image, panel (B) shows the T_2-weighted image. The solid white arrow points to the degraded portion of the patella. Panels (C–D) show the corresponding histological sections stained with safranin-O and fast green. The sub-chondral surface is demarcated with the open arrow. The PG depleted region appears at the articular surface (the superior aspect of the section) and is demarcated with a horizontal black arrow. The hyperintense region depicted on the $T_{1\rho}$-weighted image (panel A) appears to correlate with the proteoglycan deficient region on panel (C).

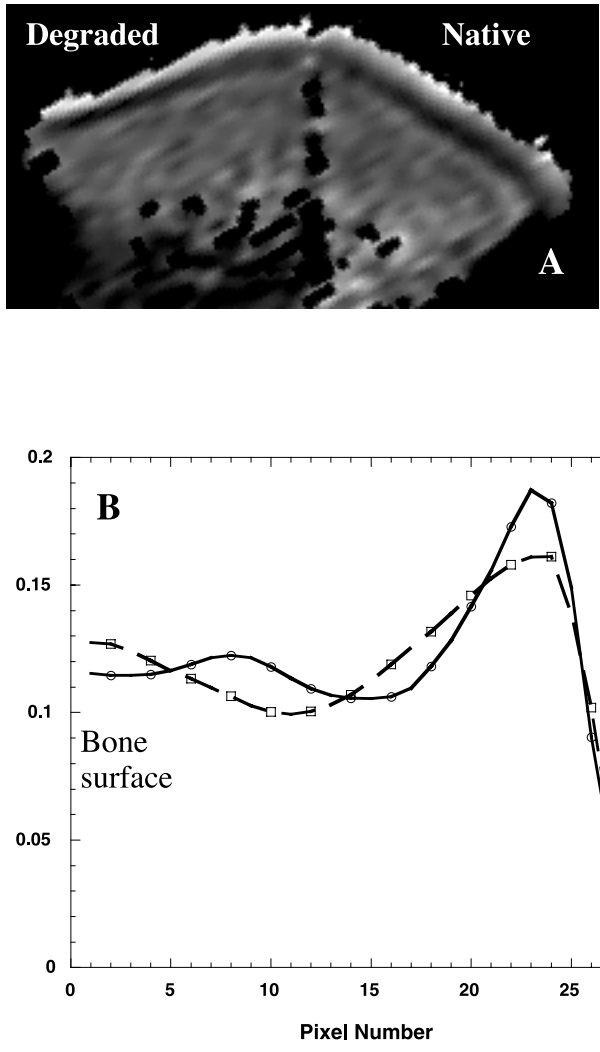

Fig. 5. Panel A shows a $T_{1\rho}$ map obtained from a patella with one half degraded with trypsin 0.2 mg/ml for 2 h. Panel B shows a plot of $T_{1\rho}$ values as a function of pixel distance from the sub-chondral bone (pixel number 0). $T_{1\rho}$ values are higher at the articular surface in the degraded side when compared to the native tissue.

MR relaxation parameters with fewer sources of error. The variation in relaxation times in native tissue was too small to account for the scatter observed in Fig. 1. For this reason, we postulate that variability in the trypsin digestion and the uncertainty in determining PG loss are major contributors to the scatter in Fig. 1. The data presented in this paper show that increased $T_{1\rho}$ is more correlated to loss of PG from the extra-cellular matrix than T_1 or T_2 relaxation. Furthermore, the linear regression analysis indicates that $T_{1\rho}$ measurements account for the majority of the variance in estimating PG loss. This indicates that serial $T_{1\rho}$ measurements are sufficient for the longitudinal detection of PG loss, and the incorporation of T_1 and T_2 measurements only marginally improves the estimation.

Prolongation of T_2 has been described in animal models of OA[29]. Our data showed that T_2 values did not demonstrate a clear trend with increasing degradation. Similar results have been reported in the literature[13]. T_1 values have not been found to be correlated with PG content in human articular cartilage[30]. We feel that the $T_{1\rho}$ technique will be a more suitable for longitudinal studies of arthritic patients.

It is worthy to note that the experimental protocol applied for this paper assumes that the tissue explants are not synthesizing PG while undergoing MR investigation or during the trypsin digestion. Hence, the accumulation of PG/hydroxyproline in the media is a direct result of the loss of PG from the tissue. Similarly, by measuring the PG after the final degradation we can compute the fractional loss. Bashir *et al*. showed that degradation of bovine cartilage with trypsin does induce a predictable pattern of PG loss[31]. The sequential digestion protocol employed in this paper may be used to effect a fractional loss of PG from the tissue.

The hydroxyproline measurement shows that the total collagen loss was less than 1% after 2 h of trypsin digestion. This result indicates that PG loss from the extra-cellular matrix is primarily responsible for the prolongation of $T_{1\rho}$ relaxation. However, it is possible that more substantial collagen degradation could also affect the $T_{1\rho}$ relaxation properties. Further experiments that investigate the effects of collagen depletion are needed to explicitly define the relative contributions of collagen and PG loss on $T_{1\rho}$ relaxation measurements.

The imaging data presented here demonstrate that $T_{1\rho}$ imaging can clearly demonstrate as little as a 15% loss of PG. Although the spectroscopic data seem to show that the $T_{1\rho}$ relaxation is not substantially altered below 20% degradation, these data represent the global average relaxation times for the entire sample. The spatially localized imaging data, on the other hand, clearly show a hyperintense region consistent with degradation changes with 15% PG loss. Therefore, the spectroscopic data presented here should be viewed as being the lower limit for the sensitivity of this technique.

The correlation observed between the hyperintense region on the $T_{1\rho}$-weighted images and the PG depleted region observed on histology provides further evidence that $T_{1\rho}$ relaxation can be exploited to generate contrast that is sensitive to the PG content of cartilage. Since the PG degradation induced by trypsin is not uniform, the contrast values determined by the region-of-interest analysis (which includes signal contributions from non-degraded tissue) must be interpreted carefully. Similarly, the spectroscopic relaxation time measurements eliminate the spatial heterogeneity in relaxation times induced by PG loss.

Olivier *et al*. have shown that the MR signal characteristics of bovine articular cartilage are spatially dependent[32]. This may be due to regional variation in the collagen and proteoglycan content in the tissue. It is therefore important to evaluate the regional variation of $T_{1\rho}$ relaxation in articular cartilage in order to establish the clinical utility of $T_{1\rho}$ imaging.

The results of this study demonstrate that $T_{1\rho}$ relaxation changes are correlated with PG loss *in vitro*. $T_{1\rho}$ MRI may thus be developed into a useful adjunct to existing techniques for the evaluation of cartilage disease. Therefore, $T_{1\rho}$ imaging has the potential to enable investigators to monitor the response to novel therapies by performing serial MRI examinations of the same patient.

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References

1. van de Loo AA, Arntz OJ, Otterness IG, van den Berg WB. Proteoglycan loss and subsequent replenishment in articular cartilage after a mild arthritic insult by IL-1 in mice: impaired proteoglycan turnover in the recovery phase. Agents Actions 1994;41:200–8.
2. Recht MP, Resnick D. MR imaging of articular cartilage: current status and future directions. Am J Roent 1994;163:283–90.
3. McCauley TR, Disler DG. MR imaging of articular cartilage. Radiology 1998;209:629–40.
4. Kneeland JB. Magnetic-resonance-imaging—Biophysical basis for the proton magnetic-resonance appearance of normal cartilage—other magnetic-resonance techniques. J Rheumat 1995;22:60–1.
5. Loeuille D, Olivier P, Mainard D, Gillet P, Netter P, Blum A. Review: magnetic resonance imaging of normal and osteoarthritic cartilage. [Review] [60 refs]. Arthritis Rheum 1998;41:963–75.
6. Bashir A, Gray ML, Boutin RD, Burstein D. Glycosaminoglycan in articular cartilage: *in vivo* assessment with delayed $Gd(DTPA)^2$—enhanced MR imaging. Radiology 1997;205:551–8.
7. Reddy R, Insko EK, Noyszewski EA, Dandora R, Kneeland JB, Leigh JS. Sodium MRI of human articular cartilage in vivo. Magn Reson Med 1998;39: 697–701.
8. Duvvuri U, Reddy R, Patel SD, Kaufman JH, Kneeland JB, Leigh JS. T1rho-relaxation in articular cartilage: effects of enzymatic degradation. Magn Reson Med 1997;38:863–7.
9. Duvvuri U, Charagundla SR, Kudchodkar SB, Kaufman JH, Kneeland JB, Rizi R, *et al*. Human Knee: In vivo $T_{1\rho}$-weighted imaging at 1.5T: preliminary experience. Radiology 2001;220:822–6.
10. Akella SVS, Regatte R, Gougoutas AJ, Borthakur A, Shapiro EM, Kneeland JB, *et al*. Proteoglycan-induced changes in $T_{1\rho}$-relaxation of articular cartilage at 4T. Magn Reson Med 2001;46:419–23.
11. Farndale RW, Sayers CA, Barrett AJ. A direct spectrophotometric microassay for sulfated glycosaminoglycans in cartilage cultures. Conn Tiss Res 1982;9:247–8.
12. Bank RA, Krikken M, Beekman B, Stoop R, Maroudas A, Lafeber FP, *et al*. A simplified measurement of degraded collagen in tissues: application in healthy, fibrillated and osteoarthritic cartilage. Matrix Biology 1997;16:233–43.
13. Borthakur A, Shapiro EM, Beers J, Kudchodkar S, Kneeland JB, Reddy R. Sensitivity of MRI to proteoglycan depletion in cartilage: comparision of sodium and proton MRI. Osteoarthris Cart 2000;8:288–93.
14. Poptani H, Duvvuri U, Mancuso A, Frazier N, Leigh JS, Glickson JD, *et al*. $T_{1\rho}$ imaging of murine brain tumors. Acad Radiol 2001;8:42–7.
15. Shapiro EM, Borthakur A, Dandora R, Kriss A, Leigh JS, Reddy R. Sodium visibility and quantitation in intact bovine articular cartilage using high field ^{23}Na MRI and MRS. J Magn Reson 2000;142:24–31.
16. Hargreaves BA, Gold GE, Lang PK, Conolly SM, Pauly JM, Bergman G, *et al*. MR imaging of articular cartilage using driven equilibrium. Magn Reson Med 1999;42:695–703.
17. Andrasko J. Water in agarose gels studied by nuclear magnetic resonance relaxation in the rotating frame. Biophys J 1975;15:1235–43.
18. Bevington PR, Robinson DK. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill 1992.
19. Jones GP. Spin-lattice relaxation in the rotating frame: weak collision case. Phy Rev 1965;148:332–5.
20. Redfield AG. Nuclear magnetic resonance saturation and rotary saturation in solids. Phys Rev 1955;98: 1787–809.
21. Markkola AT, Aronen HJ, Paavonen T, Hopsu E, Sipila LM, Tanttu JI, *et al*. Spin lock and magnetization transfer imaging of head and neck tumors. Radiology 1996;200:369–75.
22. Knispel RR, Thompson RT, Pintar MM. Dispersion of proton spin-lattice relaxation in tissues. J Magn Reson 1974;14:44–51.
23. Sridharan KR, Schreiner LJ, Kydon DW, Pintar MM, Inch WR. Characterization of normal and malignant

mouse tissue by NMR lineshape-relaxation correlations in the rotation frame. Magn Reson Med 1985;2:73–80.
24. Virta A, Komu M, Kormano M. T1rho of protein solutions at very low fields: dependence on molecular weight, concentration, and structure. Magn Reson Med 1997;37:53–7.
25. Duvvuri U, Goldberg AD, Kranz JK, Hoang L, Reddy R, Wehrli FW, *et al*. Water magnetic relaxation dispersion in biological systems: the contribution of proton exchange and implications for the non-invasive detection of cartilage degradation. Proceedings of the National Academy of Sciences USA 2001; 98:12479–84.
26. Meiboom S. Nuclear magnetic resonance study of the proton transfer in water. J Chem Phys 1965;34: 375–88.
27. Nugent A, Johnson G. Microscopic susceptibility variation and $T_{1\rho}$. Proc Int Soc Magn Reson Med 2000;1:512.
28. Bloembergen N, Purcell EM, Pound RV. Relaxation effects in NMR absorption. Phys Rev 1948;73:679.
29. Watson PJ, Carpenter TA, Hall LD, Tyler JA. Cartilage swelling and loss in a spontaneous model of osteoarthritis visualized by magnetic resonance imaging. Osteoarthritis Cart 1996;4:197–207.
30. Bashir A, Gray ML, Hartke J, Burstein D. Nondestructive imaging of human cartilage glycosaminoglycan concentration by MRI. Magn Reson Med 1999;41: 857–65.
31. Bashir A, Gray ML, Burstein D. Gd-DTPA^{2-} as a measure of cartilage degradation. Magn Reson Med 1996;36:665–73.
32. Olivier P, Loeuille D, Watrin A, Walter F, Etienne S, Netter P, *et al*. Structural evaluation of articular cartilage: potential contribution of magnetic resonance techniques used in clinical practice. Arthritis Rheum 2001;44:2285–95.